FIRE EFFECTS ON HABITAT QUALITY FOR WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) WITHIN THE CROSS TIMBERS ECOREGION

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CHAPTER I

WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) TISSUE $\delta^{13}C$ AND $\delta^{15}N$ RESPONSE TO PRESCRIBED FIRE

ABSTRACT

Stable carbon isotope ratios ($\delta^{13}C$) are linked to the photosynthetic pathways, C_3 vs. C_4, and used to construct the dietary composition of herbivores. Stable nitrogen isotope ratios ($\delta^{15}N$) are linked to nutritional condition. I assessed the response of $\delta^{13}C$ and $\delta^{15}N$ in white-tailed deer to fire at 3 Wildlife Management Areas (WMA) in Oklahoma: Lexington WMA, Okmulgee Game Management Area (GMA), and Cherokee WMA (Public Hunting Area and GMA). Seasonally representative tissues, e.g., liver (early winter), muscle (autumn), and hoof (summer), and life-span representative tissues, e.g., bone collagen, and incisor enamel, were collected from white-tailed deer harvested at the 3 WMAs during controlled hunt programs. Tissues also were collected from white-tailed deer at State-operated hunter check stations to represent of white-tailed deer from private lands. Mean $\delta^{13}C$ values of all five tissue types were within the published range of C_3 plants suggesting seasonal and life-span forage consumption is dominated by C_3 forages. Mean muscle $\delta^{15}N$ increased at the 3 WMAs between years following the application of fire. Mean hoof $\delta^{15}N$ did not differ between years at the 3 WMAs, but slight increases

were observed at Okmulgee GMA. Significant increases in mean bone $\delta^{15}N$ were observed at the 3 WMAs as mean years since fire (YSF) decreased in the units in which white-tailed deer were harvested. Mean incisor $\delta^{15}N$ did not differ between years at each WMA, but Okmulgee GMA was significantly higher than Cherokee WMA. Mean liver $\delta^{15}N$ was significantly lower at Cherokee WMA in both years of this study than Okmulgee GMA and Lexington WMA, and a significant increase was observed at Okmulgee GMA following application of fire. Greater YSF means were at Cherokee Public Hunting Area (14.4 YSF, 2008, and 14.5 YSF, 2009) and Cherokee GMA (7.4 YSF, 2008, and 12.4 YSF, 2009) and the least YSF means at Lexington WMA (2.4 YSF, 2008, and 1.75 years, 2009) and YSF means at Okmulgee GMA were in between (5.6 years, 2008, and 4.5 years, 2009). A strong correlation existed between mean YSF at each WMA and mean incisor $\delta^{15}N$ and mean hoof $\delta^{15}N$.

Key Words: carbon isotopes, Cross Timbers, nitrogen isotopes, *Odocoileus virginianus*, Oklahoma, white-tailed deer, years since fire,

INTRODUCTION

Use of naturally occurring stable isotopes in nutritional research, particularly carbon (^{13}C) and nitrogen (^{15}N), has increased in the recent decades (Roth and Hobson 2000). Stable isotopes in an animal's tissues have been linked to those of its diet (DeNiro and Epstein 1978; 1981, Tieszen et al. 1983, Tieszen and Boutton 1989, Roth and Hobson 2000). Processes involved in the incorporation of dietary nutrients and their incorporation into an animal's tissues are continually undergoing investigation (Wolf et

al. 2009). Different tissues represent isotopic incorporation from different times; i.e., liver tissue reflects more recent nutrient uptake than muscle, based on varying turnover rates (Boutton et al. 1984, Wolf et al. 2009). Metabolically active tissues (e.g., liver, muscle) have shorter turnover rates and reflect a dietary shift in a shorter time period than less metabolically active tissues (e.g., hoof, bone—Tieszen et al. 1983, Sponheimer et al. 2003, Sare et al. 2005, Walter 2006); however, Wolf et al. (2009) speculated that isotopic turnover rates of tissues were related to protein turnover and isotopic incorporation of individual tissues and may be affected by protein uptake and activity levels of an organism. Analysis of several tissue types should be conducted to understand isotopic turnover rates (DeNiro and Epstein 1981, Dalerum and Angerbjorn 2005, Walter 2006).

Levels of isotopes in muscle and liver are considered short-term nutritional indices, about 1 month and 1 week respectively, and hoof is a long-term nutrition index, about 6–12 months (Sponheimer et al. 2003, Walter 2006). Collagen is believed to closely reflect the isotopic composition of an herbivore's diet because of increased amino-acid synthesis (Krueger and Sullivan 1984). Collagen is considered a major source of carbon for isotopic analysis because it contains about 45% carbon (Krueger and Sullivan 1984). Turnover of bone tissues is the slowest of all tissues in the body that have been studied (Krueger and Sullivan 1984).

The ultimate source of carbon for all living organisms is carbon dioxide in the atmosphere (Smith and Smith 2001). Heterotrophs, like white-tailed deer (*Odocoileus virginianus*), consume necessary carbon from plant materials that produce simple sugars and starches during photosynthesis (Smith and Smith 2001). Simple sugars and starch in diets, along with digestible sugars produced by bacterial decomposition, satisfy most of

the energy requirements of an herbivore (Krueger and Sullivan 1984). The principal reason for varying carbon-isotope ratios ($^{13}C/^{12}C$; expressed as $\delta^{13}C$ in ‰ notation) in plants is the result of their differing C_3 and C_4 photosynthetic pathways (Cerling and Harris 1999). Plants that use the C_3 pathway of photosynthesis discriminate heavily on ^{13}C, which results in more deviation from the standard (Peedee Belemnite marine fossil limestone formation from South Carolina), averaging -27‰ (range: -35 to -21‰; Boutton et al. 1984, Kelly 2000, Cerling et al. 2004). Plants that use the C_4 pathway of photosynthesis discriminate less against ^{13}C and average -13‰ (range: -14 to -10‰; Boutton et al. 1984, Kelly 2000, Walter 2006). Stable isotopic ratios of $\delta^{13}C$ are enriched 1–2‰ in consumer tissues through the process of fractionation (Kelly 2000, Darr and Hewitt 2008, Cerling and Harris 1999), but the low enrichment value of ^{13}C isotopes allows them to be traced through the food web more easily than ^{15}N isotopes (Kelly 2000, Cerling and Harris 1999).

Nitrogen is a primary constituent of protein, a building block of all living material (Smith and Smith 2001). Nitrogen within the atmosphere is converted to ammonia and nitrates by bacteria, cyanobacteria, and nodule-bearing plants (Smith and Smith 2001). The highest quality plant food for herbivores is high in nitrogen (Smith and Smith 2001, Mattson 1980). The heavy isotope of nitrogen (^{15}N) is incorporated into tissues of consumer and hence results in enrichment of the nitrogen-isotope ratio ($^{15}N/^{14}N$: expressed as $\delta^{15}N$) within each trophic level (Kelly 2000). The $\delta^{15}N$ isotope ratios are difficult to trace through a food web because they vary widely in terrestrial plants (-8–18‰) and are derived from large variations in soil values and the systematic enrichment of deep-rooted plants compared to shallow roots (Kelly 2000).

White-tailed deer inhabit a variety of forest, rangeland, and agricultural areas in North and South America (Smith 1991, Heffelfinger 2006). White-tailed deer are ruminant herbivores with a rumen, reticulum, omasum, and abomasum that allow them to digest plants with microbial symbionts (Fulbright and Ortega-S. 2006, Verme and Ullrey 1984). White-tailed deer usually are classified as browsers but consume a variety of foods including grasses, sedges, fruits, nuts, forbs, mushrooms, and parts of shrubs and trees (Verme and Ullrey 1984, Smith 1991). The microbial activities within the rumen allow white-tailed deer to digest forage of low quality (Verme and Ullrey 1984). In times when availability of nutritious forage is limited, white-tailed deer rely on lipid reserves and endogenous protein (Delgiudice et al. 1990).

Habitat requirements of white-tailed deer include water, food, and thermal and protective cover (Fulbright and Ortega-S. 2006, Masters et al. 2007). Fulbright and Ortega-S. (2006) stated that optimum habitat of white-tailed deer consisted of a mosaic of woody and herbaceous plants, and diverse vegetative habitats are more resilient to harsh conditions. White-tailed deer benefit from natural disturbances and anthropogenic disturbances that mimic natural disturbance, e.g., logging, clearing, and agriculture, which create openings and early successional habitat (Smith 1991). A key to managing good white-tailed deer habitat is determining limiting factors within the habitat (Fulbright and Ortega-S. 2006, Masters et al. 2007). Diets of white-tailed deer within the Cross Timbers consist of browse and mast in the autumn and winter, and forbs and grasses in the spring and summer (Soper et al. 1993). Deer body size, changes in body mass, reproduction, and survival are determined through energy and nutrient requirements,

foraging and digestive efficiencies, and food abundances and qualities (Parker et al. 1999).

Within the Cross Timbers ecoregion of Oklahoma, home ranges of white-tailed deer average about 100 ha (Leslie et al. 1996). Leslie et al. (1996) found that both sexes of white-tailed deer selected areas treated by prescribed fire during summer months and avoided untreated areas during summer and autumn because of decreased forage quality. Burning can alter abundance and quality of forage available to white-tailed deer (Soper et al.1993, Zimmerman et al. 2006). Zimmerman et al. (2006) found that white-tailed deer and mule deer (*Odocoileus hemionus*) benefitted nutritionally from a patchy burn pattern within 3 years postfire in South Dakota. Correlations between dietary nitrogen and fecal nitrogen concentrations have been demonstrated (Leslie et al. 2008), and high concentrations of fecal nitrogen are associated with high-quality diets (Leslie and Starkey 1985, Osborn and Ginnett 2001).

Prescribed fire can be used to improve and enlarge habitats for wild ungulates and therefore enhance their nutrition (Hobbs and Spowart 1984). When conducted under proper conditions, fire can benefit white-tailed deer by increasing production of browse (Dills 1970). The application of prescribed burning on a given landscape can vary by season, length of fire-return interval, and severity depending on management goals for that landscape (Fuhlendorf and Engle 2001, Van Lear and Harlow 2002). In forests, an increase in fire frequency promotes growth of grasses because they can recover relatively quickly after a burn; fires tend to reduce woody vegetation and fuel loads within the understory (Van Lear and Harlow 2002). Prescribed fires are affected by environmental

conditions such as soil moisture, humidity, and fuel moisture (Collins and Barber 1985, Groninger 2005).

Many Wildlife Management Areas (WMA) in Oklahoma have established prescribed burning regimes to benefit wildlife habitat quality. As the use of prescribed fire spreads to differing ecosystems, it is important to understand effects of fires on vegetation and hence wildlife populations. When conducting prescribed fire, the management goal determines the return interval of fire. Effect of frequency of prescribed burns has been studied in the tall-grass prairie, Ozark deciduous forests, and southern pine forests (Adams et al. 1982, Masters et al. 1993, Masters et al. 1996, Sparks et al. 2002), but little research has been conducted on whether or not prescribed burning in the Cross Timbers ecoregion is nutritionally advantageous to wildlife. I used stable isotope ratios of carbon (^{13}C) and nitrogen (^{15}N) to compare the nutritional signature of white-tailed deer that forage on three WMAs owned by the Oklahoma Department of Wildlife Conservation and privately owned land within the Cross Timber ecoregion. I tested the hypothesis that prescribed burning had a positive nutritional effect on white-tailed deer as reflected in a predicted negative correlation between years since fire and nutritional outcomes. I also assessed correlations among tissue isotopic values.

STUDY AREA

My project was conducted along longitudinal and latitudinal gradients to represent the diversity of Cross Timbers ecosystems in eastern and central Oklahoma. Part of this project occurred on Lexington (35.1° N, 97.2° W), Cherokee (35.8° N, 95.1° W), and Okmulgee (35.6° N, 96.1° W) WMAs. Lexington WMA is located about 30 km

southeast of Norman, Oklahoma; it encompasses 3,849.5 ha (9,512 acres) and is near the western edge of the Cross Timbers ecoregion and the transition into grassland habitats. Cherokee WMA is located about 19 km east of Ft. Gibson, Oklahoma, along the Cherokee and Muskogee county borders; it is composed of 12,691.2 ha (31,360 acres) representing the eastern edge of the Cross Timbers ecoregion where it meets the oak-hickory forests of the Ozark Mountains. Cherokee WMA is divided into two sections: the northern section (6,475.1 ha) open to public hunting (PHA) and the southern Game Management Area (GMA; 6,216.1 ha) used for controlled hunts. Okmulgee WMA is about 8 km west of Okmulgee, Oklahoma; it is 4,411.2 ha (10,900 acres) located in the central part of the Cross Timbers ecoregion. Okmulgee WMA also is divided into PHA and a GMA. The GMA comprises the majority of the WMA, and public hunting areas are located on the northeastern side of the Deep Fork River and to the northwest of the GMA. My study also included areas near Dickson (34.2° N, 97.0° W), Coalgate (34.5° N, 96.2° W), Hartshorne (34.8° N, 95.6° W), and Claremore (36.3° N, 95.6° W), Oklahoma. The diversity of study sites presumably reflected varied nutrient availability to white-tailed deer.

The Cross Timbers ecoregion is a complex mosaic of vegetation considered to be a western extension of the Ozark Plateau (Garrison et al. 1977, Stahle et al. 1996). The Cross Timbers extends from central Texas through eastern and central Oklahoma and into southeastern Kansas (Duck and Fletcher 1945, Kuchler 1964). The relatively dry climate and sandy soils of the Cross Timbers prevent trees from attaining large sizes, which reduces their value for timber production (Clark and Hallgren 2003, Roe 1998). Post oaks (*Quercus stellata*), one of two dominate canopy tree species, rarely exceed 15 m in

height and 60 cm in diameter (Therrel and Stahle 1998). The Cross Timbers may be the least disturbed forest ecosystem and contains the largest stands of old-growth forest surviving in the eastern United States (Clark and Hallgren 2003, Stahle et al. 1996).

Typical Cross Timbers is dominated by post oak and blackjack oak (*Quercus marilandica*), intermixed with oak-savannas and glade communities (Clark and Hallgren 2004, Kuchler 1964, Roe 1998, Stahle et al. 1996). Oak savannas consist of oak species intermixed with grasslands that are dominated by fire-adapted species (e.g., little bluestem [*Schizachyrium scoparium*], big bluestem [*Andropogon gerardii*], indiangrass [*Sorghastrum nutans*]; Smith and Smith 2001). Annual forbs tend to grow in the gaps among bunchgrasses (Collins and Barber 1985) and can constitute 44% of annual diets of white-tailed deer (Gee et al. 1991). Forbs and browse can be found on the forest floor and make up 41% of white-tailed deer annual diets (Gee et al. 1991). The forest can include 200–500-year-old post oaks that dominate dry, rocky positions and occur in soils with higher percentages of sand and eastern red cedar (*Juniper virginiana*) up to 600 years old along rock outcroppings (Stahle et al. 1996, Therrell and Stahle 1998). These are the oldest oak and cedar trees currently known in Oklahoma and are, no doubt, among the oldest trees still present in the south central United States (Stahle et al. 1996, Therrell and Stahle 1998).

MATERIALS AND METHODS

Tissue samples for white-tailed deer were collected at each study location, opportunistically during annual white-tailed deer hunts and when hunters check-in their deer. Tissue samples were taken during controlled hunts on the three WMAs (October–

December) and at student-operated State deer check stations during the opening weekend of the deer gun season (22–23 November). A hind hoof and leg bone were taken from each white-tailed deer processed by cutting off the leg, with a reciprocating saw, just below the metatarsal gland. About 2 g of muscle were collected from the sternum, about 5 mm inside the incision to avoid collecting hair, fatty tissues, and dirt (Walter 2006, Walter and Leslie 2009). An incisor was collected from each lower jaw, which was removed for aging. During controlled hunts on the WMAs, a sample of liver was collected from participating hunters or by retrieving gut piles. All tissue samples were stored frozen prior to being oven-dried at 70°C to a constant weight (Walter 2006): about 72 hours for muscle and liver and 48 hours for bone, incisor, and hoof. The location (using an Oklahoma Atlas & Gazetteer or WMA paper map), sex, and age of the harvested white-tailed deer were recorded.

Dried muscle and liver tissue samples were ground through a 1-mm mesh screen in a Wiley mill. Despite previous concerns (Peterson and Fry 1987, Kelly 2000), Walter and Leslie (2009) found no effect of lipid content in muscle samples from Rocky Mountain elk (*Cervus elaphus*) on $\delta^{13}C$ ($P = 0.522$) and $\delta^{15}N$ ($P = 0.967$) isotopic estimates; therefore, lipids were not removed from muscle samples. Hoof and bone samples were cleaned with alcohol, filed to remove collagen of the bone and the exterior of the hoof, and then dried to a constant weight. Hoof samples were filed at the middle one-third of the hoof to represent summer diet (Walter and Leslie 2009). Dried incisors were crushed using a mortar and pestle, separating the enamel from the dentin, and pieces of enamel for analysis were removed using tweezers. Twenty samples (10 male and 10 female, when possible) of each tissue from each sampling location were analyzed. About

2 mg of each sample were placed into a 5- x 9-mm tin capsule and analyzed for carbon and nitrogen isotopes by an Isotope Ratio Mass Spectrometer (IRMS) at the University of California–Davis (Walter 2006, Walter and Leslie 2009). Blind replicates of various tissue samples were submitted to document laboratory precision.

Levene's test for homogeneity was used to insure sample data were balanced and random. Data were analyzed with PROC GLM in the Statistical Analyst Systems software (SAS; SAS Institute Inc. 2003) for main effects and interaction by area and sex. One-way analysis of variance (ANOVA), MEANS statement, was used to determine year effect in the five tissues for the two years sampled. A LSMEANS statement was used for two-way ANOVA comparison of year-area and YSF-area interactions. Tukey's multiple comparison tests were used to identify differences between area means. Correlation analyses were used (SAS Institute Inc. 2003) to assess relationships between carbon and nitrogen isotopes for each tissue, carbon and nitrogen isotope values between tissues, and YSF and nitrogen isotope values.

RESULTS

Tissue samples from 257 white-tailed deer were collected from hunts in 2008 (n = 168) and 2009 (n = 89). Samples in 2008 were collected from Cherokee WMA (Cherokee GMA and Cherokee PHA), Okmulgee GMA, Lexington WMA, Hartshorne, Dickson, Coalgate, Claremore, and Thunderbird State Park. Samples in 2009 were collected from the three WMAs and Hartshorne. One hundred ninety six muscle, 255 hoof, 255 bone, 147 incisor, and 111 liver samples were analyzed for isotopic ratios of carbon and nitrogen. Means ± standard error (*SE*) of seasonally representative tissues

(liver, muscle, hoof) are reported in Table 1, and means ± *SE* of tissues representing average life-span of white-tailed deer (bone, incisor) are reported in Table 2.

Because a one-way ANOVA showed an effect of year for all tissue isotope values, except hoof $\delta^{15}N$ ($F_{1,253} = 2.09$, $P = 0.150$), bone $\delta^{13}C$ ($F_{1,253} = 0.03$, $P = 0.869$), and liver $\delta^{15}N$ ($F_{1,109} = 0.01$, $P = 0.908$), areas from both years were analyzed separately. A year-area interaction occurred for muscle $\delta^{13}C$ ($F_{3,185} = 5.62$, $P = 0.001$), muscle $\delta^{15}N$ ($F_{3,185} = 7.42$, $P < 0.001$), hoof $\delta^{13}C$ ($F_{4,241} = 2.64$, $P = 0.035$), hoof $\delta^{15}N$ ($F_{4,241} = 2.54$, $P = 0.041$), bone $\delta^{13}C$ ($F_{4,241} = 2.97$, $P = 0.020$), and liver $\delta^{15}N$ ($F_{2,104} = 11.14$, $P < 0.001$), but no year-area interaction occurred for liver $\delta^{13}C$ ($F_{2,104} = 0.18$, $P = 0.833$), incisor $\delta^{15}N$ ($F_{3,138} = 1.00$, $P = 0.394$), incisor $\delta^{13}C$ ($F_{3,138} = 1.24$, $P = 0.298$), and bone $\delta^{15}N$ ($F_{4,241} = 1.29$, $P = 0.274$; Tables 1 and 2). The 2009 Okmulgee mean muscle $\delta^{15}N$ ($\bar{x} = 5.75‰ \pm 0.87$ *SE*) differed significantly from the 2009 Hartshorne sample ($P < 0.001$) and from all other samples ($P < 0.001$). Mean $\delta^{15}N$ in 2009 Okmulgee liver samples was significantly different from 2008 Okmulgee, 2008 and 2009 Lexington, and 2008 and 2009 Cherokee GMA ($P < 0.001$ for each).

No area-sex interaction occurred for muscle $\delta^{13}C$ ($F_{4,135} = 0.75$, $P = 0.562$), muscle $\delta^{15}N$ ($F_{4,135} = 0.76$, $P = 0.555$), hoof $\delta^{13}C$ ($F_{4,134} = 0.57$, $P = 0.688$), hoof $\delta^{15}N$ ($F_{4,134} = 1.55$, $P = 0.192$), bone $\delta^{13}C$ ($F_{4,135} = 1.13$, $P = 0.344$), bone $\delta^{15}N$ ($F_{4,135} = 0.75$, $P = 0.560$), incisor $\delta^{13}C$ ($F_{4,115} = 0.05$, $P = 0.995$), incisor $\delta^{15}N$ ($F_{4,115} = 0.76$, $P = 0.556$), liver $\delta^{13}C$ ($F_{3,87} = 0.40$, $P = 0.753$), and liver $\delta^{15}N$ ($F_{3,87} = 0.46$, $P = 0.760$). Okmulgee male white-tailed deer had higher mean muscle $\delta^{15}N$ than either sex at Lexington ($P < 0.001$), Cherokee GMA ($P < 0.001$), and Cherokee PHA ($P < 0.001$) but did not differ from females at Okmulgee ($P = 0.972$). Males at Cherokee GMA and Cherokee PHA

had lower mean muscle $\delta^{15}N$ than either sex at Lexington ($P < 0.001$) and Okmulgee ($P < 0.001$) but did not differ from females at Cherokee GMA or Cherokee PHA.

No correlation was observed between $\delta^{13}C$ and $\delta^{15}N$ for all tissues with the exception of a slight correlation in hoof ($r^2 = 0.156$, $P < 0.001$, $n = 254$). Carbon isotopic ratios ($\delta^{13}C$) of individual tissues were positively correlated with each other and significant among liver, hoof, and muscle ($P < 0.001$; Figure 1a–c). When correlations were performed using $\delta^{13}C$ means of liver, hoof, and muscle for each area, mean hoof $\delta^{13}C$ and mean muscle $\delta^{13}C$ were significantly correlated ($r^2 = 0.506$, $P = 0.014$; Figure 1e). Correlations between mean liver $\delta^{13}C$ and mean muscle $\delta^{13}C$ ($r^2 = 0.326$, $P = 0.069$) and mean liver $\delta^{13}C$ and mean hoof $\delta^{13}C$ ($r^2 = 0.294$, $P = 0.209$) were positively related but not significant (Figure 1d and f). There was a positive correlation among individual tissue $\delta^{15}N$ values for liver, muscle, and hoof ($P < 0.001$; Figure 2a–c). Correlations for mean liver $\delta^{15}N$ and mean muscle $\delta^{15}N$ ($r^2 = 0.80$, $P = 0.007$; Figure 2e) and hoof $\delta^{15}N$ and liver $\delta^{15}N$ ($r^2 = 0.916$, $P < 0.001$; Figure 2f) increased in strength from correlations of individual tissue $\delta^{15}N$ values. The correlation between mean hoof $\delta^{15}N$ and mean muscle $\delta^{15}N$ remained as strong as individual tissue $\delta^{15}N$ values (Figure 2d).

A negative correlation occurred between mean YSF of each area and mean $\delta^{15}N$ values from each WMA for liver ($r^2 = 0.335$, $P = 0.229$) and muscle ($r^2 = 0.330$, $P = 0.137$; Figure 3a and b). Mean hoof $\delta^{15}N$ (i.e., reflecting summer diets) from each WMA had a strong negative correlation with mean YSF ($r^2 = 0.780$, $P = 0.004$; Figure 3c). A negative correlation also occurred between mean YSF and mean bone $\delta^{15}N$ values ($r^2 = 0.268$, $P = 0.189$; Figure 4a). Mean incisor $\delta^{15}N$ values (i.e., life-span diets) from each

WMA had a strong negative correlation with mean YSF (r^2 = 0.637, P = 0.018; Figure 4b).

DISCUSSION

Plants that use the C_3 pathway of photosynthesis tend to be less enriched in $\delta^{13}C$, averaging -27‰ (range: -35 to -21‰), and plants that use the C_4 pathway of photosynthesis (PEP Carboxylase) average -13‰ (range: -14 to -10‰; Bender 1971, Boutton et al. 1984, Cerling and Harris 1999, Kelly 2000, Walter 2006). C_3 plants tend to possess more negative values in areas of closed canopies but can vary due to light intensity, nutrient availability, water stress, and temperature (Farquhar et al. 1989, Cerling et al. 2004, Feranec 2007). Tissues from Cherokee GMA and Cherokee PHA tended to have the most negative $\delta^{13}C$ ratios which may have been due to increased midstory and higher canopy cover resulting in lower light levels and reduced CO_2 within white-tailed deer browsing heights (Farquhar et al. 1989, Feranec 2007). Cerling et al. (2004) showed that extreme ^{13}C depletion exists in closed canopy forest between the canopy and the forest floor with values varying as much as 10‰. Other studies have shown a depletion of ^{13}C and accounted for the depletion by two effects: isotopic enrichment of CO_2 at the ground level due to limited exchange with the free troposphere and increased fractionation of $^{13}C/^{12}C$ at very low photosynthetic rates at low light levels (Medina and Minchin 1980, Medina et al. 1986, Van der Merwe and Medina 1989, Farquhar et al. 1989, Cerling et al. 2004).

Tissues with relatively short turn-over rates (i.e., liver, feces, and muscle) represent short-term temporal differences in diet compared with tissues with long turn-

over rates or inert tissues, such as hoof, bone, and incisor (DeNiro and Epstein 1978, Teiszen et al. 1983, Walter 2006). Mean liver and muscle $\delta^{13}C$ ranged from -28.38 to -25.84‰ suggesting that white-tailed deer diets during autumn and early winter consisted mainly of C_3 plants. Liver $\delta^{13}C$ differed from muscle $\delta^{13}C$ suggesting that diets during autumn and early winter varied in their composition. To build fat reserves for winter, white-tailed deer diets in the autumn in the Cross Timbers largely consist of acorns (8–23%, or more depending on availability), along with browse (Gee et al. 1991, Soper et al. 1993). Grasses begin to increase in white-tailed deer diets in autumn and forbs decrease in diets as they begin to senesce (Gee et al. 1991). Muscle $\delta^{13}C$ differed between Lexington WMA and Cherokee GMA and Cherokee PHA. Lexington WMA represented the western portion of the Cross Timber sampling range, where the Cross Timbers transitions into the tall-grass prairie, and had more open areas than Cherokee WMA. Cherokee WMA represented the eastern part of the Cross Timbers sampling range, where the Cross Timbers transitions into the eastern deciduous forests.

Keratin is laid down during the growth of white-tailed deer hooves and its isotope signatures represent forage quality at the time it was laid down; and it generally becomes inert relative to turnover (Walter 2006). Hoof $\delta^{13}C$ was consistently higher during both years in the northeastern part of the study area (i.e., Cherokee GMA, Cherokee PHA, and Okmulgee) of sampling, which could be related to rainfall patterns or vegetative cover densities. Thunderbird had the highest hoof $\delta^{13}C$ (-24.31‰ ± 1.42 SE) and differed significantly from Lexington, Okmulgee, and Cherokee. Thunderbird was sampled to represent an unmanaged area receiving no fire within at least the past 10 years. Student check stations from Dickson, Coalgate, and Hartshorne also differed from the three

management areas. Mean hoof $\delta^{13}C$ ranged from -24.31 to -26.61‰ suggesting summer diets in white-tailed deer were composed of C_3 plants. Hoof $\delta^{13}C$ differed from liver $\delta^{13}C$ and muscle $\delta^{13}C$ indicating summer diets differed from autumn and early winter diets. Summer diets of white-tailed deer within the Cross Timbers consist mainly of forbs and some browse (Gee et al. 1991, Soper et al. 1993).

Plants high in nitrogen are considered high-quality herbivore forage, important for growth, reproduction, etc. (Mattson 1980). The $\delta^{15}N$ in tissues of ungulates, such as white-tailed deer, depends on the amount nitrogen content of their forage (Deniro and Epstein 1981). Plants can acquire nitrogen through bacteria, cyanobacteria, and nodules; however, plants that acquire nitrogen by fixing atmospheric nitrogen ($\delta^{15}N = 0‰$) tend to be less enriched in $\delta^{15}N$ (Virginia and Delwiche 1982, Ambrose and DeNiro 1986, Hobbie et al. 2000, Walter 2006). Tissues of herbivorous species that forage in open grasslands are typically more enriched in $\delta^{15}N$ than individuals of the same species foraging in a closed forest (Ambrose 1991, Walter 2006).

A significant increase in muscle $\delta^{15}N$ between years existed for Okmulgee GMA, Lexington WMA, and Cherokee PHA. Okmulgee GMA also exhibited a significant increase in liver $\delta^{15}N$ between years. Yearly differences between tissue isotope ratios within the same area may be related to the effects of annual variation in temperatures, rainfall (i.e., increase $\delta^{15}N$ with decreasing precipitation; Cormie and Schwarz 1996, Feranec 2007), or prescribed burning on forages. Okmulgee GMA, Lexington WMA, and Cherokee PHA all experienced prescribed burns in March between sample years, and Cherokee GMA, which did not differ significantly between years, did not have a prescribed burn during the sampling period.

Hoof $\delta^{15}N$ did not differ between years for any areas. Prescribed fire in late winter (February–March) can either not significantly change forb cover (Brockway et al. 2002) or significantly change forb cover the following year (Thompson et al. 1991) depending on the environmental conditions during the burn. The ability of forb cover to not change post-fire may explain the lack of difference in hoof $\delta^{15}N$ between years, because hoof isotopic values should represent summer forage consumption when forbs are dominant in white-tailed deer diets. Hoof $\delta^{15}N$ was the lowest at Cherokee GMA and PHA during both years, but only differed significantly from the 2009 Okmulgee GMA sample. Seedling stem densities of woody herbaceous species increase after late winter and summer fires in the form of resprouts and new growth (Adams et al. 1982). This increase in woody seedlings provides an increase in available browse to white-tailed deer (Dills 1970). The increase in young seedlings post fire may account for increases in muscle and liver $\delta^{15}N$ because seedlings and new growth of woody herbaceous species can contain higher concentrations of nitrogen and soluble protein (Fleck et al. 1996).

Bone and incisor isotopic values reflect the averaged nutrition of an animal over its lifespan (Halley et al. 2006); average age of white-tailed deer in this study was 2.5 years. Over all areas, mean bone $\delta^{13}C$ (range: -22.45 to -24.05) and mean incisor δ13C (range: -22.78 to -24.29) were within the range of C_3 plants, which means white-tailed deer diets consist mainly of C_3 forage throughout a lifespan. Bone $\delta^{15}N$ from 2008 Cherokee GMA and 2008 Cherokee PHA were lower than all other areas. Incisor $\delta^{15}N$ was the lowest at Cherokee WMA, which follows Ambrose (1991) for species within a closed forest being less enriched in nitrogen than the same species in a more open habitat.

Levels of $\delta^{13}C$ and $\delta^{15}N$ were not correlated in my study, as they were in previous studies (Hobson et al. 2000, Walter and Leslie 2009). Hobson et al. (2000) hypothesized that a correlation between $\delta^{13}C$ and $\delta^{15}N$ may be stronger in species that consume primarily meat than in species that consume primarily plants because of positive fractionation of isotopic values from one trophic level to another. This lack of correlation may be due to the differences in scale of previous studies and my study (i.e., which spanned central and eastern Oklahoma), differences in soil types, and/or precipitation gradients. Cherokee GMA and PHA soils are porous gravel soils, Okmulgee soils are dominantly sandy-loam, and Lexington soils are mainly clay soils. Soils consisting of fine particles tend to retain nutrients longer than more coarse soils. Further research should focus on geographical correlations of $\delta^{13}C$ and $\delta^{15}N$, perhaps based on soil types or rainfall patterns. Isotopic values can vary within the same species between areas based on nutrient availability to plants and photosynthetic rates (Virginia and Delwiche 1982, Peterson and Fry 1987, Evans 2001, Walter 2006). Kelly (2000) also suggested that nitrogen isotope ratios may be influenced by rainfall patterns; terrestrial mammals in xeric habitats may have higher $\delta^{15}N$ than in mesic habitats.

A significant positive correlation existed for $\delta^{15}N$ values among all tissues (Figure 2a–e). Hoof $\delta^{15}N$ (summer) explained 67% of the variation in muscle $\delta^{15}N$ (autumn) and 58% of the variation in liver $\delta^{15}N$ (early winter), suggesting that when diets at the beginning of the growing season were low in protein, they continued to remain low in protein through the rest of the year. Hoof $\delta^{15}N$ differed from muscle $\delta^{15}N$ and liver $\delta^{15}N$, and muscle $\delta^{15}N$ differed from liver $\delta^{15}N$ (Table 1). These differences can be explained

by the consumption of different forage species during different seasons of the year and/or by the use of differing habitats during the year.

Leslie et al. (1996) found that white-tailed deer in the Cross Timbers will select or avoid habitats during particular seasons in response to prescribed burning. Correlations between YSF and mean tissue $\delta^{15}N$ showed negative relationship for all tissue types, suggesting fire enhanced nutritional outcomes for white-tailed deer. Soper et al. (1993) found that alterations to nutritional quality of white-tailed deer browse were apparent 5–6 years after treatment in central Oklahoma Cross Timbers, and Zimmerman et al. (2006) found white-tailed deer benefit nutritionally within 3 years post-fire in South Dakota. Mean tissue $\delta^{15}N$ was highest 4–5 years since fire (Figures 3 and 4). Available soil nitrogen (NH_4^+ and NO_3^-) is important to recovery of primary productivity and plant growth following fire because it can be readily taken up and assimilated (Raison 1979, Wan et al. 2001). Wan et al. (2001) found NH_4^+ increased significantly (199%) immediately post-fire before returning to pre-fire levels after 1 year, and NO_3^- lagged before increasing over 300% within 12 months post-fire then returned to pre-fire levels within 5 years. Foliar nitrogen content has been shown to decrease immediately following fire and increase for at least four growing seasons post-fire (Landberg et al. 1984). When time since fire remains low, nitrogen content within plants declines, and in tall-grass prairie burned annually, plant nitrogen concentrations are lower than unburned areas (Blair 1997). Lexington WMA (mean years since fire = 2.4 and 1.75 for 2008 and 2009, respectively); overall mean $\delta^{15}N$ values were lower than Okmulgee GMA (mean years since fire = 5.6 and 4.5 for 2008 and 2009, respectively), but only differed for incisor and liver. The difference in overall mean liver $\delta^{15}N$ may reflect increased winter

nutrition in white-tailed deer when fire return intervals are near 4–5 years. Overall mean $\delta^{15}N$ values for all tissues at Okmulgee GMA and Lexington WMA were higher than Cherokee GMA (YSF means = 7.4 and 12.4 for 2008 and 2009, respectively) and Cherokee PHA (YSF means = 14.4 and 14.5 for 2008 and 2009, respectively), which did not differ from each other.

White-tailed deer within the Cross Timbers benefitted from fire with increases of $\delta^{15}N$ values in all tissue types with relation to mean YSF of an area. A strong relationship existed between mean years since fire and mean hoof $\delta^{15}N$ of an area. Hoof $\delta^{15}N$ represented diets consumed during summer and increases in hoof $\delta^{15}N$ reflected increased protein uptake. Significant increases in muscle tissue $\delta^{15}N$ were observed the year following a fire corresponding to increases in protein uptake when white-tailed deer are building fat reserves prior to winter. White-tailed deer also tended to benefit in protein uptake from a fire return interval of 4–5 years within the Cross Timbers. A fire return interval of 4–5 years maintains available browse to white-tailed deer and maximizes the use of available nitrogen within the system. Hoof, muscle, and liver isotopic values provide seasonal indices to white-tailed deer nutrition because there is enough variation in $\delta^{13}C$ and $\delta^{15}N$ to represent differences in forage. Bone and incisor isotopic values are representative of the averaged life-span diet of white-tailed deer; however, it is important to remember that estimation of assimilated diet from stable isotopes depends on the accurate estimation of enrichment between the diet and tissue (Pearson et al. 2003, Robbins et al. 2005). Whenever specific enrichment values are not available for the species of study, a researcher must either use published, interspecific

means, measurements in other species, or develop predictive regressions (Robbins et al. 2005).

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Table 1. Means (±SE) for isotopic concentrations of metabolically active tissue samples: hoof, muscle, and liver, representing dietary intake during summer, autumn and early winter. Tissues collected during controlled hunts from four State managed areas and during opening weekend of white-tailed deer gun season at student operated check stations, 2008 and 2009. Sexes were combined in sample size. Concentrations with different letters within a column differed at $P < 0.05$.

	Hoof (n=13–20)				Muscle (n=13–20)				Liver (n=10–20)			
Area	δ^{13}C (SE)		δ^{15}N (SE)		δ^{13}C (SE)		δ^{15}N (SE)		δ^{13}C (SE)		δ^{15}N (SE)	
2008												
Lexington WMA	-25.20 (0.14)	CDEFGH	3.52 (0.28)	BCDEFGH	-26.52 (0.17)	BCDEF	2.79 (0.21)	DEFG	-26.59 (0.25)	ABCDE	3.83 (0.26)	B
Okmulgee GMA	-25.77 (0.12)	ABCDEF	3.52 (0.30)	BCDEFGH	-26.96 (0.19)	BCDEF	2.92 (0.22)	CDEFG	-27.35 (0.20)	ABC	4.24 (0.20)	B
Cherokee GMA	-26.48 (0.17)	ABC	2.46 (0.20)	DEFGHI	-27.22 (0.21)	ABCDE	1.63 (0.14)	FGHI	-27.12 (0.15)	ABCD	2.32 (0.34)	C
Cherokee PHA	-26.61 (0.15)	ABC	1.81 (0.22)	FGHI	-26.70 (0.15)	BCDEF	1.28 (0.17)	GHI				
Claremore	-25.41 (0.28)	BCDEFG	5.09 (0.52)	ABCD	-26.44 (0.42)	BCDEF	3.92 (0.51)	BCD				
Dickson	-24.46 (0.18)	DEFGH	4.52 (0.23)	ABCDEF								
Coalgate	-24.77 (0.15)	DEFGH	4.42 (0.23)	ABCDEFG								
Hartshorne	-24.57 (0.28)	DEFGH	3.10 (0.28)	CDEFGHI								
Thunderbird SP	-24.31 (0.39)	EFGH	3.06 (0.22)	BCDEFGHI	-25.84 (0.30)	DEF	2.37 (0.17)	DEFGHI	-26.30 (0.40)	BCDE	3.57 (0.23)	B
2009												
Lexington WMA	-25.25(0.12)	BCDEFGH	3.78(0.31)	ABCDEFGH	-26.36(0.22)	CDEF	3.91(0.22)	BCD	-25.87(0.15)	CDE	3.85(0.22)	B
Okmulgee GMA	-26.18(0.12)	ABCDE	4.67(0.39)	ABCDEF	-26.70(0.17)	BCDEF	5.75(0.22)	A	-26.70(0.14)	ABCDE	6.17(0.22)	A
Cherokee GMA	-26.59(0.12)	ABC	1.95(0.23)	FGHI	-27.66(0.31)	ABCD	2.48(0.14)	DEFGH	-26.65(0.10)	ABCDE	2.23(0.14)	C
Cherokee PHA	-26.17(0.21)	ABCDE	1.68(0.28)	FGHI	-28.38(0.44)	ABC	2.57(0.22)	DEFGH				
Hartshorne	-25.37(0.22)	BCDEFG	3.83(0.36)	CDEFGHI	-26.34(0.34)	CDEF	4.21(0.30)	BC				

Table 2. 2008 and 2009 means (±SE) for isotopic concentrations of metabolically inert tissues, bone and incisor, representing life-time nutrition. Sexes were combined in sample size. Concentrations with different letters within a column differed at $P < 0.05$.

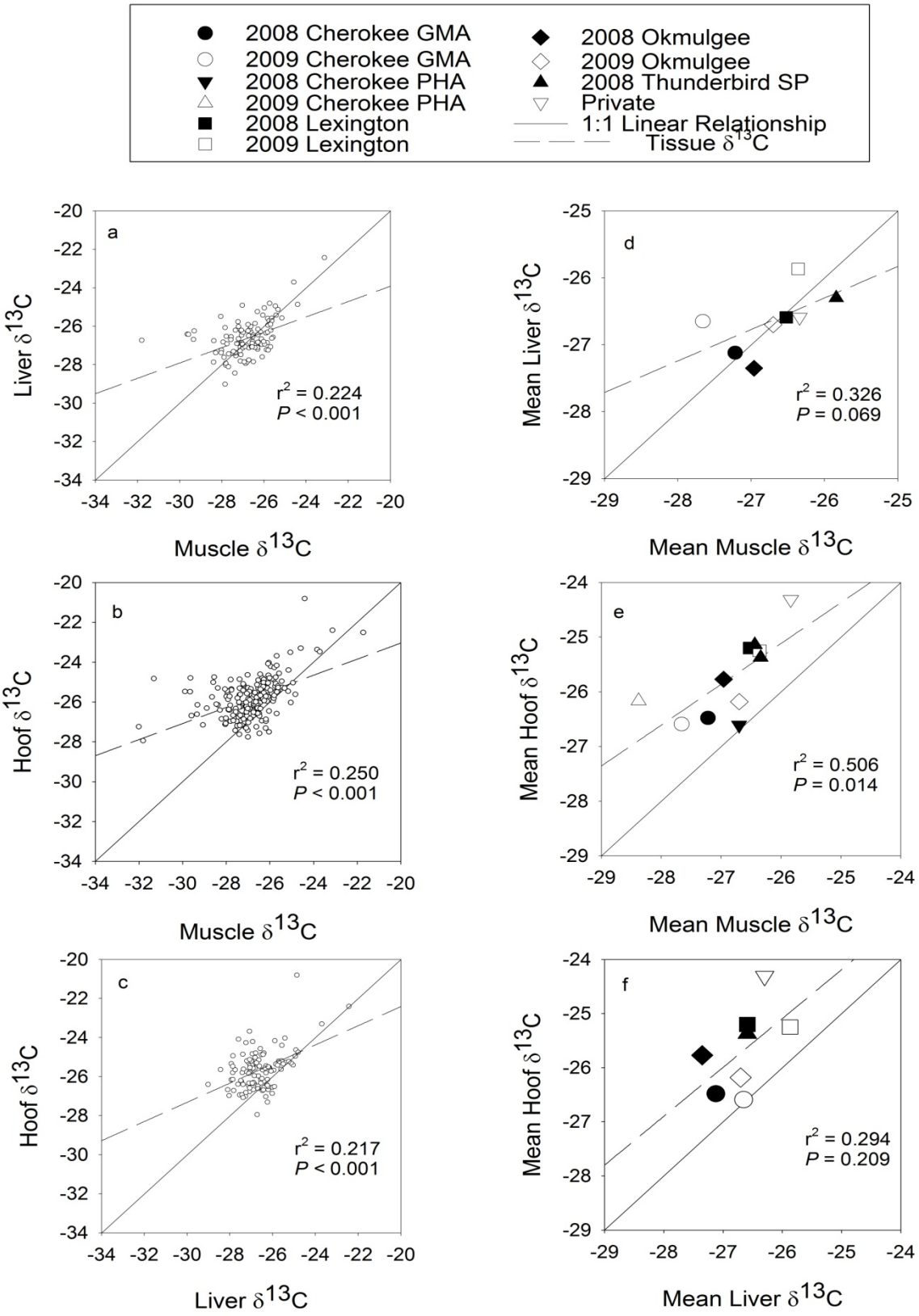
	Bone (n=13–20)				Incisor (n=8–20)			
Area	$\delta^{13}C$ (SE)		$\delta^{15}N$ (SE)		$\delta^{13}C$ (SE)		$\delta^{15}N$ (SE)	
2008								
Lexington WMA	-23.49 (0.17)	ABCDEFGH	3.18 (0.26)	DEFGH	-22.78 (0.32)	BC	4.13 (0.25)	BCD
Okmulgee GMA	-23.56 (0.16)	ABCDEFG	3.36 (0.23)	CDEFGH	-23.18 (0.22)	ABC	4.78 (0.30)	ABC
Cherokee GMA	-24.05 (0.23)	ABCDE	1.88 (0.18)	HI	-23.63 (0.49)	ABC	3.28 (0.21)	CDE
Cherokee PHA	-23.58 (0.18)	ABCDEFG	1.45 (0.21)	HI	-22.90 (0.28)	ABC	3.05 (0.36)	CDE
Claremore	-23.67 (0.22)	ABCDEF	4.50 (0.39)	ABCDEF	-23.31 (0.55)	ABC	5.51 (0.41)	AB
Dickson	-22.63 (0.14)	DEFGHI	4.17 (0.21)	BCDEFG				
Coalgate	-22.62 (0.23)	CEFGHI	4.31 (0.16)	BCDEFG				
Hartshorne	-22.65 (0.15)	CEFGHI	3.11 (0.35)	DEFGH				
Thunderbird SP	-22.65 (0.31)	BCDEFGHI	2.66 (0.23)	EFGHI				
2009								
Lexington WMA	-22.53(0.13)	FGHI	4.61(0.23)	ABCDE	-22.83(0.22)	BC	4.11(0.24)	BCD
Okmulgee GMA	-23.91(0.22)	ABCDE	5.69(0.37)	ABC	-24.29(0.14)	AB	5.12(0.31)	ABC
Cherokee GMA	-23.83(0.15)	ABCDE	3.21(0.20)	DEFGH	-23.95(0.29)	ABC	3.02(0.28)	CDE
Cherokee PHA	-23.53(0.20)	ABCDEFGH	3.55(0.32)	BCDEFGH	-23.39(0.37)	ABC	2.62(0.30)	DE
Hartshorne	-22.45(0.27)	FGHI	4.81(0.38)	ABCDE				

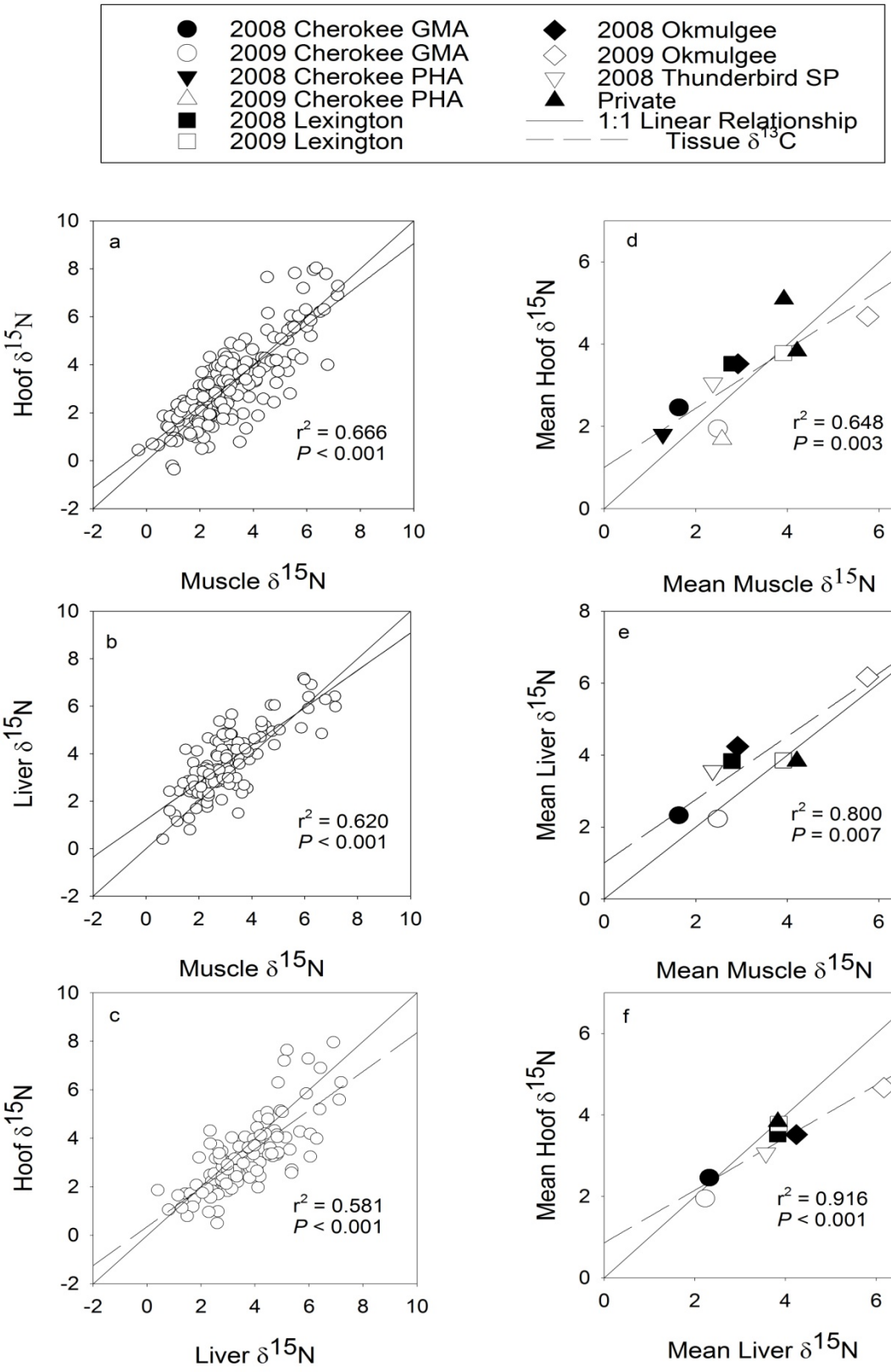
Figure 1. Correlations of a) individual muscle and liver $\delta^{13}C$, b) individual muscle and hoof $\delta^{13}C$, c) individual liver and hoof $\delta^{13}C$, d) mean muscle and mean hoof $\delta^{13}C$, e) mean muscle and mean liver $\delta^{13}C$, f) mean liver and mean hoof $\delta^{13}C$ from white-tailed deer collected at Lexington Wildlife Management Area, Okmulgee Game Management Area, Cherokee Public Hunting Area, Cherokee Game Management Area, and student check-stations (Private) during 2008 and 2009. 1:1 relationship line is included for reference.

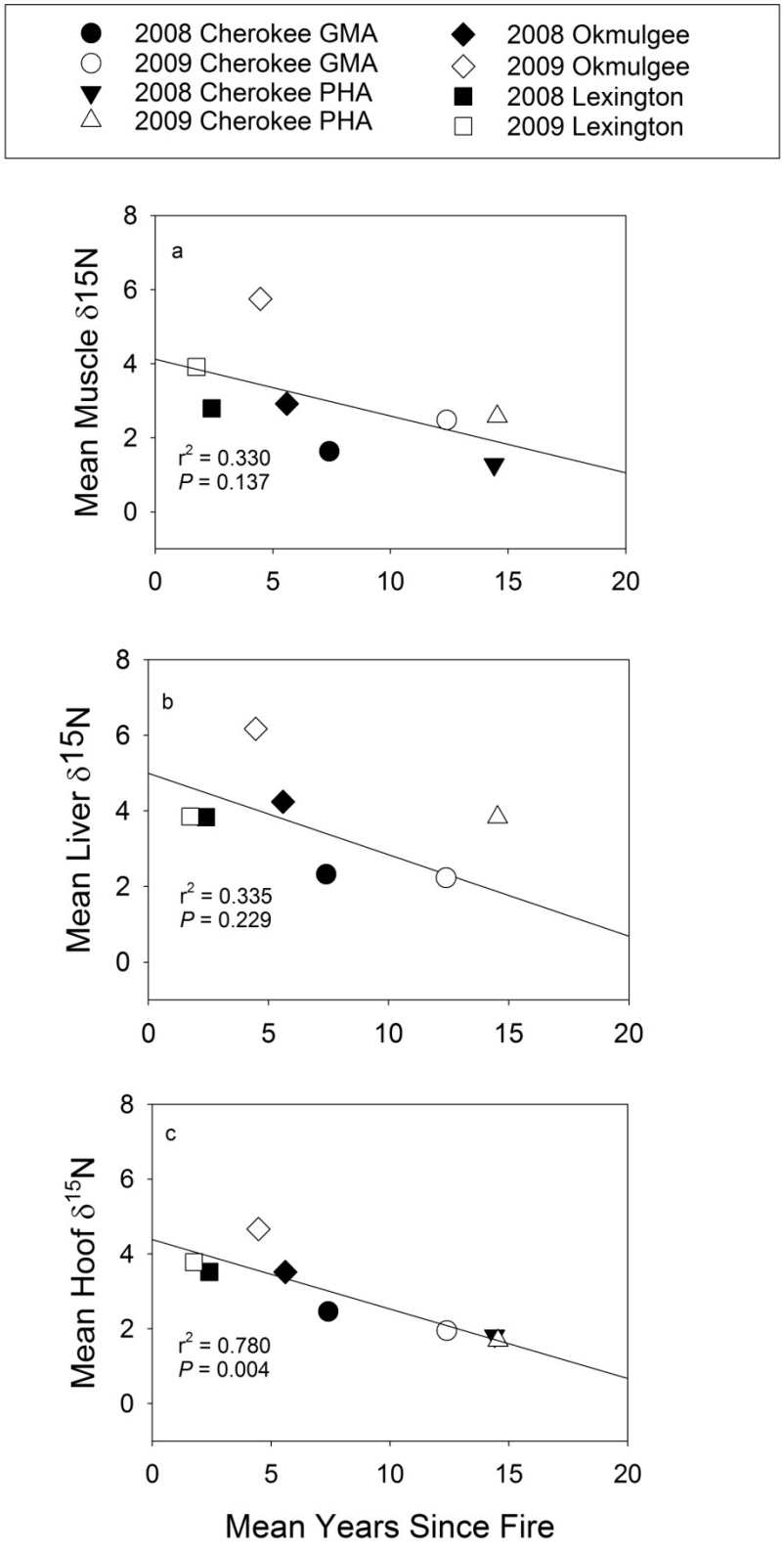
Figure 2. Correlations of a) individual muscle and liver $\delta^{15}N$, b) individual muscle and hoof $\delta^{15}N$, c) individual liver and hoof $\delta^{15}N$, d) mean muscle and mean hoof $\delta^{15}N$, e) mean muscle and mean liver $\delta^{15}N$, f) mean liver and mean hoof $\delta^{15}N$ from white-tailed deer collected at Lexington Wildlife Management Area, Okmulgee Game Management Area, Cherokee Public Hunting Area, Cherokee Game Management Area, and student check-stations (Private) during 2008 and 2009. 1:1 relationship line is included for reference.

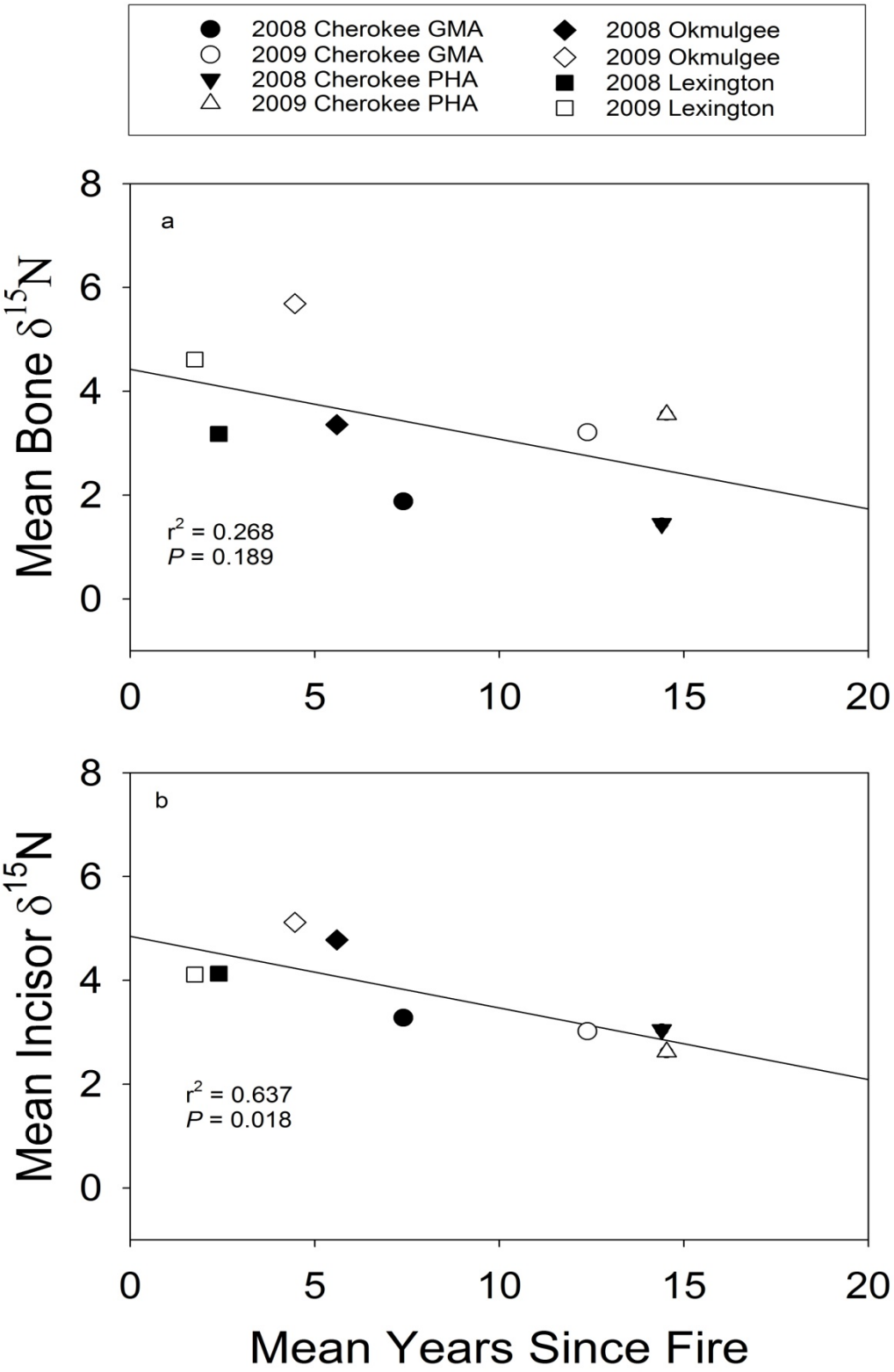
Figure 3. Correlations of seasonally representative tissues a) mean muscle $\delta^{15}N$ (autumn) and mean years since fire (YSF), b) mean liver $\delta^{15}N$ (early winter) and mean years since fire, c) mean hoof $\delta^{15}N$ (summer) and years since fire collected from Lexington Wildlife Management Area, Okmulgee Game Management Area, Cherokee Public Hunting Area, and Cherokee Game Management Area during 2008 and 2009.

Figure 4. Correlations of life-span representative tissues a) mean bone $\delta^{15}N$ and years since fire (YSF), and b) mean incisor $\delta^{15}N$ and years since fire collected from Lexington Wildlife Management Area, Okmulgee Game Management Area, Cherokee Public Hunting Area, and Cherokee Game Management Area during 2008 and 2009.


2008 Cherokee GMA
2009 Cherokee GMA
2008 Cherokee PHA
2009 Cherokee PHA
2008 Lexington
2009 Lexington
2008 Okmulgee
2009 Okmulgee
2008 Thunderbird SP
Private
1:1 Linear Relationship
Tissue δ13C
a
Liver δ13C
Muscle δ13C
r2 = 0.224
P < 0.001
b
Hoof δ13C
Muscle δ13C
r2 = 0.250
P < 0.001
c
Hoof δ13C
Liver δ13C
r2 = 0.217
P < 0.001
d
Mean Liver δ13C
Mean Muscle δ13C
r2 = 0.326
P = 0.069
e
Mean Hoof δ13C
Mean Muscle δ13C
r2 = 0.506
P = 0.014
f
Mean Hoof δ13C
Mean Liver δ13C
r2 = 0.294
P = 0.209


2008 Cherokee GMA
2009 Cherokee GMA
2008 Cherokee PHA
2009 Cherokee PHA
2008 Lexington
2009 Lexington
2008 Okmulgee
2009 Okmulgee
2008 Thunderbird SP
Private
1:1 Linear Relationship
Tissue δ13C
a
Hoof δ15N
Muscle δ15N
r2 = 0.666
P < 0.001
b
Liver δ15N
Muscle δ15N
r2 = 0.620
P < 0.001
c
Hoof δ15N
Liver δ15N
r2 = 0.581
P < 0.001
d
Mean Hoof δ15N
Mean Muscle δ15N
r2 = 0.648
P = 0.003
e
Mean Liver δ15N
Mean Muscle δ15N
r2 = 0.800
P = 0.007
f
Mean Hoof δ15N
Mean Liver δ15N
r2 = 0.916
P < 0.001


2008 Cherokee GMA
2009 Cherokee GMA
2008 Cherokee PHA
2009 Cherokee PHA
2008 Okmulgee
2009 Okmulgee
2008 Lexington
2009 Lexington
a
Mean Muscle δ15N
r2 = 0.330
P = 0.137
b
Mean Liver δ15N
r2 = 0.335
P = 0.229
c
Mean Hoof δ15N
r2 = 0.780
P = 0.004
Mean Years Since Fire


2008 Cherokee GMA
2009 Cherokee GMA
2008 Cherokee PHA
2009 Cherokee PHA
2008 Okmulgee
2009 Okmulgee
2008 Lexington
2009 Lexington
a
Mean Bone δ15N
r2 = 0.268
P = 0.189
b
Mean Incisor δ15N
r2 = 0.637
P = 0.018
0
2
4
6
8
0
5
10
15
20
Mean Years Since Fire

CHAPTER II

NUTRITIONAL CONDITION OF LATE-WINTER WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) USING STABLE CARBON AND NITROGEN ISOTOPE RATIOS AND NITROGEN IN FECES

ABSTRACT

Fecal sampling is a convenient non-intrusive method of assessing the nutrition of herbivores with positive correlations demonstrated between fecal and dietary nitrogen. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes ratios have been used to construct the composition of C_3 and C_4 plants within an herbivore's diet and infer nutritional quality. I assessed nutritional condition of white-tailed deer (*Odocoileus virginianus*) in the Cross Timbers ecoregion using stable isotopes of carbon and nitrogen and nitrogen in feces during late winter. Fecal samples were collected opportunistically in February–March, 2009 and 2010 from white-tailed deer at 3 Wildlife Management Areas (WMA) in Oklahoma: Lexington WMA, Okmulgee Game Management Area, and Cherokee Game Management Area. Fecal nitrogen concentrations at the 3 WMAs suggested white-tailed deer consumed relatively high-quality diets in late winter. Fecal $\delta^{13}C$ levels at the WMAs reflected late winter diets dominant in C_3 plants. Depleted fecal $\delta^{15}N$ levels suggested that white-tailed deer may consume browse when more nutritious

forage was limited. Despite variability in the application of prescribed fire at the 3 WMAs, low values of years since fire within theoretical home ranges in combination with levels of fecal $\delta^{13}C$, fecal $\delta^{15}N$, and fecal nitrogen suggested that white-tailed deer at the 3 WMAs used comparable, and presumably optimal, home ranges in late-winter with similar nutrition.

Key Words: white-tailed deer, carbon isotopes, nitrogen isotopes, fecal nitrogen, Oklahoma, Cross Timbers, *Odocoileus virginianus*

INTRODUCTION

Many nutritional indices have been used to assess the physiological condition of white-tailed deer (*Odocoileus virgianus*; Verme and Ullrey 1984, Osborn and Ginnett 2001). Protein content of the diet has been shown to be related to blood-urea nitrogen concentrations (Verme and Ullrey 1984) and fecal nitrogen (N) concentrations (Leslie et al. 2008, Osborn and Ginnett 2001). Fecal N is a common nutritional index that has been shown to be positively correlated with dietary intake, dietary digestibility, seasonal dietary quality, dietary protein, and weight changes in ruminants (Cordova et al. 1978, Leslie and Starkey 1985, 1987, Walter 2006, Leslie et al. 2008), and high concentrations of fecal N are associated with high-quality diets (Leslie and Starkey 1985, Osborn and Ginnett 2001). The influence of protein-precipitating compounds can make seasonal comparisons in fecal N difficult (Hobbs 1987, Leslie and Starkey 1987, Osborn and Jenks 1998, Walter 2006), although this may be less of a concern in mixed diets (Leslie et al. 2008) Differences in digestibility can make microhistological analysis of gut contents or

feces unreliable because they can over- or underestimate forages consumed (Leslie et al. 1983, Hobbs and Spowart 1984, Osborn et al. 1997, Servello et al. 2005, Walter 2006). Fecal N can be a valuable index of dietary quality and nutritional status when the relationship between dietary nitrogen and fecal N are established for a ruminant in a given location (Leslie and Starkey 1985, Leslie et al. 2008). Fecal indices are a noninvasive alternative to study diet quality and are often used because they do not require disturbance, stress, or death of wild ruminants (Leslie and Starkey 1985, Howery and Pfister 1990, Osborn and Ginnett 2001).

Isotopic ratios of carbon ($^{13}C/^{12}C$; expressed as $\delta^{13}C$ in ‰ notation) and nitrogen ($^{15}N/^{14}N$: expressed as $\delta^{15}N$) have been used to identify dietary intake of C_3 and C_4 forage and the nutritional condition of herbivores (Tieszen et al. 1983, Kelly 2000, Sponheimer et al. 2003a, Walter 2006, Walter and Leslie 2009). The C_3 plants discriminate heavily on ^{13}C, which results in more deviation from the standard (Peedee Belemnite marine fossil limestone formation from South Carolina), averaging -27‰ (range: -35 to -21‰; Boutton et al. 1984, Kelly 2000, Cerling et al. 2004, Walter 2006). The C_4 plants discriminate less against ^{13}C and average -13‰ (range: -14 to -10‰; Boutton et al. 1984, Kelly 2000, Walter 2006). Nitrogen in the atmosphere is converted to ammonia and nitrates by bacteria, cyanobacteria, and nodule-bearing plants (Smith and Smith 2001). The highest quality plant food for herbivores is high in digestible nitrogen (Smith and Smith 2001, Mattson 1980). Ungulates grazing on a higher proportion of C_3 forage are expected to have higher nutritional quality (Wilson and Hattersley 1989, Post et al. 2001, Walter 2006). Ratios of $\delta^{15}N$ isotopes are difficult to trace through a food web because they vary widely in terrestrial plants (-8–+18‰) and are derived from large variations in

soil values and the systematic enrichment of deep-rooted plants over those with shallow roots (Kelly 2000). Seasonal and habitat differences can be reflected in carbon and nitrogen isotopes in ungulates (Teiszen and Imbamba 1980, Tieszen et al. 1989, Walter 2006).

It is understood that isotopic concentrations of different animal tissues can represent isotopic incorporation and nutrient uptake from differing times; e.g., liver tissue reflects more recent nutrient uptake than muscle, based on varying rates of isotopic ^{15}N incorporation into animal tissues (Boutton et al. 1984, Wolf et al. 2009; see Chapter I). Tissue turnover rates have been hypothesized to be related to the metabolism of the tissue (Tieszen et al. 1983) and/or the protein uptake and activity levels of an individual (Wolf et al. 2009). Reflecting passage rates of digesta, fecal isotopic carbon and nitrogen can be used to compare ungulate diets consumed within 1–3 days (Tieszen et al. 1983, Coates et al. 1991, Walter 2006) or an integration of diets consumed over more than a week, depending on differences in digesta abilities and quality of forages consumed (Sponheimer et al. 2003b). Sponheimer et al. (2003b) found that isotopic turnover of feces was much faster in hindgut-fermenting herbivores (e.g., horses, ~60 h) than foregut-fermenting herbivores (e.g., alpacas, ~200 h).

When using fecal isotopic concentrations, it is important to understand the fractionation between diet and feces (Sponheimer et al. 2003a). Fractionation in large herbivores averaged -0.8‰ for $\delta^{13}C$ during laboratory feeding studies (range: -0.3 – -1.3; Sponheimer et al. 2003a). Depletion of $\delta^{13}C$ between diet and feces is not fully understood at present, but the fractionation is considered minimal and should not hamper

dietary reconstruction (Sponheimer et al. 2003a). Fecal $\delta^{15}N$ is typically enriched over dietary $\delta^{15}N$, and urea $\delta^{15}N$ is depleted (Sponheimer et al. 2003b, Hwang et al. 2007).

White-tailed deer inhabit a variety of habitats such as forest, rangeland, and agricultural areas (Smith 1991, Heffelfinger 2006). Leslie et al. (1996) found that home ranges of white-tailed deer averaged about 100 ha in the Cross Timbers ecoregion of north-central Oklahoma. Optimum habitat of white-tailed deer consists of a mosaic of woody and herbaceous plants, and diverse vegetative habitats are more resilient to harsh conditions (Fulbright and Ortega-S. 2006). Determining limiting factors of the habitat is key to managing good deer habitat (Fulbright and Ortega-S. 2006, Masters et al. 2007). Diets of white-tailed deer in the Cross Timbers generally consist of browse and mast in autumn and winter, and forbs and grasses in spring and summer (Soper et al. 1993). Winter diets of white-tailed deer also can contain agricultural crops (e.g., wheat and rye) and are considered important winter forages when available (Gee et al. 1991). Body size, changes in body mass, reproduction, and survival of white-tailed deer are determined through energy and nutrient requirements, foraging and digestive efficiencies, and food abundance and qualities (Parker et al. 1999). Winters in the Cross Timbers can vary from mild to severe (Gee et al. 1991) and can be a time when white-tailed deer are nutritionally stressed (Masters et al. 1993, Delgiudice 1995, Osborn and Jenks 1998).

White-tailed deer are ruminant herbivores with a rumen, reticulum, omasum, and abomasum that allow them to digest plants with microbial symbionts (Fulbright and Ortega-S 2006, Verme and Ullrey 1984). The species is usually classified as a browser but consume a variety of foods including grasses, sedges, fruits, nuts, forbs, mushrooms, and parts of shrubs and trees (Verme and Ullrey 1984). Microbial fermentation in the

rumen allows deer to digest forage of low quality (Verme and Ullrey 1984). In times when availability of nutritious forage is limited, deer rely on lipid reserves and endogenous protein (Delgiudice et al. 1990).

Prescribed fire can be used to improve and enlarge habitats for wild ungulates and enhance their nutrition (Hobbs and Spowart 1984). Application of prescribed burning on a given landscape can vary by season, length of fire-return interval, and severity depending on management goals for that landscape (Fuhlendorf and Engle 2001, Van Lear and Harlow 2002). In forests, an increase in fire frequency promotes growth of grasses because they can recover relatively quickly after a burn; fires tend to reduce woody vegetation and fuel loads within the understory (Van Lear and Harlow 2002). Prescribed fires are affected by environmental conditions such as soil moisture, humidity, and fuel moisture (Groninger 2005).

Burning can alter abundance and quality of forage available to white-tailed deer (Soper et al.1993, Zimmerman et al. 2006). Leslie et al. (1996) found that both sexes of deer selected areas treated by prescribed fire during summer and avoided untreated areas during summer and autumn because of decreased forage quality. Zimmerman et al. (2006) found that deer benefitted nutritionally from a patchy burn pattern within 3 years postfire in South Dakota. Hobbs and Spowart (1984) found that improvements in diet quality, as a response to prescribed burning, exceeded the improvements in individual forages of mule deer (*Odocoileus hemionus*) and mountain sheep (*Ovis canadensis*).

Many Wildlife Management Areas (WMA) in Oklahoma have established prescribed burning regimes to benefit wildlife habitat quality, and managers may use

food plots to supplement winter nutrition of white-tailed deer. Prescribed fire has become a common wildlife habitat management tool, and understanding its effect on vegetation and wildlife populations is important. Prescribed burning has been studied in the tall-grass prairie, Ozark deciduous forests, and southern pine forests (Adams et al. 1982, Masters et al. 1993, Masters et al. 1996, Sparks et al. 2002), but little research has been conducted on nutritional advantages of prescribed burning in the Cross Timbers ecoregion to white-tailed deer. I used stable isotope ratios of ^{13}C and ^{15}N in combination with fecal N concentrations to assess nutritional characteristics of late winter diets of white-tailed deer on three WMAs owned by the Oklahoma Department of Wildlife Conservation in the Cross Timbers ecoregion that received variable prescribed burning. I tested the hypotheses that 1) stable isotope ratios can be used with fecal N to differentiate nutritional characteristics of late-winter diets of white-tailed deer, 2) negative correlations existed between fecal $\delta^{13}C$ and fecal $\delta^{15}N$, fecal $\delta^{15}N$ and fecal N particularly when use of agricultural food plots was prevalent, and 3) negative correlations existed between years since fire and fecal $\delta^{15}N$ and fecal N.

STUDY AREA

My project was conducted along longitudinal and latitudinal gradients to represent the diversity of Cross Timbers ecosystems in eastern and central Oklahoma. Part of this project occurred on Lexington (35.1° N, 97.2° W), Cherokee (35.8° N, 95.1° W), and Okmulgee (35.6° N, 96.1° W) WMAs. Lexington WMA is located about 30 km southeast of Norman, Oklahoma; it encompasses 3,849.5 ha (9,512 acres) and is near the western edge of the Cross Timbers ecoregion and the transition to grassland habitats. Cherokee WMA is located about 19 km east of Ft. Gibson, Oklahoma, along the

Cherokee and Muskogee county borders; it is composed of 12,691.2 ha (31,360 acres) representing the eastern edge of the Cross Timbers ecoregion where it meets the oak-hickory forests of the Ozark Mountains. Cherokee WMA is divided into two sections: the northern section (6,475.1 ha) open to public hunting (PHA) and the southern Game Management Area (GMA; 6,216.1 ha) used for controlled hunts. Okmulgee WMA is about 8 km west of Okmulgee, Oklahoma; it is 4,411.2 ha (10,900 acres) located in the central part of the Cross Timbers ecoregion. Okmulgee WMA also is divided into PHA and a GMA. The GMA comprises the majority of the WMA, and public hunting areas are located on the northeastern side of the Deep Fork River and northwest of the GMA. Habitat diversity of study sites presumably reflected varied nutrient availability to white-tailed deer.

The 3 WMAs consist of 3 varied applications of prescribed fire. The fire frequencies (number of fires/decade) at Lexington WMA ranged from 1.3–2.2 and 2.2 fires/decade occurred in 7 of 15 units. The number of years since a unit last burned at Lexington WMA ranged from 1–6 years. Units at Okmulgee GMA had fire frequencies of 1–5 and years since fire in a given unit ranged from 1–5 with 5 years since fire occurring in 7 of 11 units. Cherokee GMA ranged in fire frequencies from 0.4–2.9 and years since fire ranged from 2–25 years. The 3 WMAs plant food plots as supplementary dietary sources during winter and generally consisted of winter wheat.

The Cross Timbers ecoregion is a complex mosaic of vegetation considered to be a western extension of the Ozark Plateau (Garrison et al. 1977, Stahle et al. 1996). The Cross Timbers extends from central Texas through eastern and central Oklahoma and into southeastern Kansas (Duck and Fletcher 1945, Kuchler 1964). The relatively dry climate

and sandy soils of the Cross Timbers prevents trees from attaining large sizes, which reduces their value for timber production (Clark and Hallgren 2003, Roe 1998). Post oaks (*Quercus stellata*), one of two dominate canopy tree species, rarely exceed 15 m in height and 60 cm in diameter (Therrel and Stahle 1998). The Cross Timbers may be the least disturbed forest ecosystem and contains the largest stands of old-growth forest surviving in the eastern United States (Clark and Hallgren 2003, Stahle et al. 1996).

Typical Cross Timbers is dominated by post oak and blackjack oak (*Quercus marilandica*), intermixed with oak-savannas and glade communities (Clark and Hallgren 2004, Kuchler 1964, Roe 1998, Stahle et al. 1996). Oak savannas consist of oak species intermixed with grasslands that are dominated by fire-adapted species (e.g., little bluestem [*Schizachyrium scoparium*], big bluestem [*Andropogon gerardii*], indiangrass [*Sorghastrum nutans*]; Smith and Smith 2001). Annual forbs tend to grow in the gaps among bunchgrasses (Collins and Barber 1985) and can constitute 44% of annual diets of white-tailed deer (Gee et al. 1991). Forbs and browse can be found on the forest floor and typically make up 41% of white-tailed deer annual diets (Gee et al. 1991). The forest can include 200–500-year-old post oaks that dominate dry, rocky positions and occur in soils with higher percentages of sand and eastern red cedar (*Juniper virginiana*) up to 600 years old along rock outcroppings (Stahle et al. 1996, Therrell and Stahle 1998). These are the oldest oak and cedar trees currently known in Oklahoma and are, no doubt, among the oldest trees still present in the south-central United States (Stahle et al. 1996, Therrell and Stahle 1998).

MATERIALS AND METHODS

Fecal sampling took place in February–March 2009 and 2010, which represented late-winter diets during mid- to late-gestation of female white-tailed deer. Fresh feces were collected opportunistically in the three WMAs by following deer trails and tracks and by examining areas where deer were spooked from feeding or bedding. Using non-powdered latex gloves, 8–12 pellets from each group were placed into brown paper bags, and the location of the sample collection was recorded with a handheld GPS unit and later plotted in ArcGIS.

Laboratory Preparation

Fecal samples were air-dried to a constant weight. Dried fecal samples were ground through a 1-mm mesh screen in a Wiley mill. About 7–20 fecal samples from each sampling location and for both years were analyzed. About 2 mg of each sample were placed into a 5- x 9-mm tin capsule and analyzed for carbon and nitrogen isotopes in an Isotope Ratio Mass Spectrometer (IRMS) at the University of California–Davis (Walter 2006, Walter and Leslie 2009). Replicates of various tissue samples were submitted to document laboratory precision.

GIS Applications

Coordinates of the locations of where fecal samples were collected were recorded as decimal degrees into an Access database and imported into ArcGIS 9.3 (ESRI 2008). Latitude and longitude were converted to UTMs in ArcGIS 9.3 to match coordinate systems with land-cover data. Land-cover data came from Natural Resource Conservation Service (2001), U.S. Department of Agriculture. A theoretical 100-ha

home range (Leslie et al. 1996) was established around each point of fecal collection. In ArcGIS 9.3, Hawth's tools, Raster Tools – Thematic Raster Summary, was used to extract cover data for each 100-ha home range, and percent cover was recorded as forest, grassland, agriculture, or miscellaneous.

Theoretical home ranges were overlaid on aerial photos of each WMA. Units from each WMA were digitized in ArcGIS 9.3 and then clipped for each fecal-sample home range. A years since fire (YSF) value was assigned to each home range according the number of years since the unit encompassing the majority of the home range was last burned.

Statistical Analysis

PROC GLM was used in the Statistical Analyst Systems software (SAS Institute Inc. 2003) to perform one-way analysis of variance (ANOVA) and determine effects of year, area, and YSF. Levene's test for homogeneity was used to insure sample data were balanced and random. When year or area data were heterogeneous, Welch's (1951) ANOVA was used to test for significance. A two-way ANOVA was used in a LSMEANS statement to determine interactions of year and area. Tukey's multiple comparison tests were used to identify differences between area means. Regression analyses were used to explore relationships between $\delta^{13}C$ and $\delta^{15}N$, $\delta^{15}N$ and fecal N, YSF and $\delta^{15}N$, and YSF and fecal N. Statistical significance was set at $P < 0.05$.

RESULTS

Fresh fecal samples were collected from 77 white-tailed deer during February–March of 2009 ($n = 25$) and 2010 ($n = 52$) at Cherokee GMA, Okmulgee GMA, and

Lexington WMA. Mean $\delta^{15}N \pm$ standard error (*SE*), mean δ13C ± *SE*, and mean fecal N ± *SE* are reported in Table 1.

Because a one-way ANOVA showed a significant year effect for fecal $\delta^{13}C$ ($F_{1,75} = 8.17$, $P = 0.006$) and $\delta^{15}N$ ($F_{1,75} = 7.98$, $P = 0.006$), year and areas were treated separately. A significant year-area interaction did not occur for fecal $\delta^{13}C$ ($F_{2,71} = 1.45$, $P = 0.240$), but it did for fecal $\delta^{15}N$ ($F_{2,71} = 15.31$, $P < 0.001$). Mean fecal $\delta^{15}N$ at Cherokee GMA was higher in 2010 than in 2009 ($P < 0.001$). Okmulgee GMA ($P = 0.641$) and Lexington WMA ($P = 0.085$) did not differ in mean fecal $\delta^{15}N$ between years. Lexington WMA and Okmulgee GMA did not differ from each other in fecal $\delta^{15}N$ in 2009 ($P = 0.939$), 2010 ($P = 0.521$), or overall ($P = 0.993$). Lexington WMA differed between years for fecal $\delta^{13}C$ ($P = 0.005$), but Cherokee GMA ($P = 0.749$) and Okmulgee GMA ($P = 0.061$) did not differ between years. No year-area interaction occurred for fecal N ($F_{2,71} = 0.06$, $P = 0.938$). Fecal N ranged from 1.76% ± 0.19 to 2.19% ± 0.26 and showed no significant differences between areas or years ($P > 0.05$; Table 1).

Theoretical home ranges, based on fecal collections, at Cherokee GMA averaged 12% agriculture, 3.4% grassland, and 65% forest in 2009; however, they averaged 0% agriculture, 12% grassland, and 73.9% forest in 2010. Theoretical home ranges at Okmulgee GMA averaged 87.1% forest, 0.6% grassland, and 0% agriculture in 2009 and 82.9% forest, 0.6% grassland, and 0% agriculture in 2010. At Lexington WMA, theoretical home ranges averaged 72.5% forest, 14.7% grassland, and 0% agriculture in 2009 and 67.4% forest, 18.8% grassland, and 0% agriculture in 2010. The percent cover of food plots could not be assessed with the scale of the cover data available.

Theoretical home ranges of Okmulgee GMA in 2010 differ from 2009 Cherokee GMA ($P < 0.001$) and 2010 Lexington WMA ($P < 0.001$) in mean percent forest cover. Theoretical home range at Okmulgee GMA in 2009 differed in mean percent forest cover from all other theoretical home ranges ($P < 0.05$), except 2010 Okmulgee GMA ($P = 0.912$). Theoretical home ranges did not differ in mean percent forest between years at Lexington WMA ($P = 0.763$) and Cherokee GMA ($P = 0.233$) and did not differ from each other in 2009 ($P = 0.526$) or 2010 ($P = 0.391$). Theoretical home ranges at Cherokee GMA differ between years in mean percent grassland cover ($P < 0.001$), but they did not differ at Lexington WMA ($P = 0.231$) and Okmulgee GMA. Mean percent grassland cover of theoretical home ranges at Cherokee GMA differ from Lexington WMA in 2009 ($P < 0.001$) and 2010 ($P < 0.001$). Theoretical home ranges at Okmulgee GMA were lower in mean percent grassland cover than Lexington WMA in 2009 ($P < 0.001$) and 2010 ($P < 0.001$) and Cherokee GMA in 2010 ($P < 0.001$). Theoretical home ranges at Cherokee GMA in 2009 were the only ones to contain agricultural land cover classifications.

No correlations of individual fecal $\delta^{13}C$ and fecal $\delta^{15}N$ values ($r^2 < 0.001$, $P = 0.899$, $n = 77$) or mean fecal $\delta^{13}C$ and mean fecal $\delta^{15}N$ values for each area ($r^2 = 0.105$, $P = 0.531$, $n = 6$) were noted. Similarly, individual fecal $\delta^{15}N$ and fecal N values ($r^2 < 0.001$, $P = 0.983$, $n = 77$) and mean fecal $\delta^{15}N$ and mean fecal N for each area ($r^2 = 0.463$, $P = 0.137$, $n = 6$) were not correlated. No correlation between YSF and fecal $\delta^{15}N$ for individual values ($r^2 = 0.035$, $P = 0.115$, $n = 72$) or mean YSF and mean fecal $\delta^{15}N$ for each area ($r^2 = 0.226$, $P = 0.341$, $n = 6$) were noted. Similarly, individual values of

fecal N and YSF (r^2 = 0.002, P = 0.684, n = 72) and mean fecal N and mean YSF for each area (r^2 = 0.076, P = 0.596, n = 6) were not correlated.

DISCUSSION

Stable carbon isotope ratios ($\delta^{13}C$) in feces have been used to assess seasonal diets of African herbivores (Codron et al. 2005, Botha and Stock 2005, Codron et al. 2007a, 2007b). In African studies, grazers fed predominately on C_4 grasses and browsers fed mostly on C_3 browse (Codron et al. 2005) making assessments of the composition of diets relatively easy. When C_3 herbaceous plants exist together with C_3 browse assessing the composition of a mixed feeding herbivore (i.e., white-tailed deer) diet can be difficult. White-tailed deer can consume a variety of forages throughout the course of the year and include forbs, grasses, browse, agricultural crops, and mast (Gee et al. 1991, Soper et al. 1993); however, seasonal and life-span $\delta^{13}C$ values from white-tailed deer tissues suggest the majority of their diets consist of mostly C_3 plants (see Chapter I).

Winter is a period when nutritional quality of diets is important to the reproductive success and survival of white-tailed deer (Osborn and Jenks 1998). Fecal collections from three WMAs in Oklahoma were performed in 2009 and 2010 at the end of winter when female white-tailed deer are in mid- to late-gestation. All fecal $\delta^{13}C$ values suggested a diet of mostly C_3 plants. The maximum difference between mean fecal $\delta^{13}C$ for the three WMAs was only 1.48‰ (range: -28.83–-30.30‰), suggesting that late-winter diets at each WMA were similar in source. Given fractionation rates between feces and diet of $\delta^{13}C$, averaging -0.8‰ for large herbivores (Sponheimer et al. 2003a), and variability within a plant species based upon varying photosynthetic rates

and available CO_2, it is plausible that some of my study samples reflected isotopic composition of mostly planted food plots. Food plots are often used to supplement winter nutrition of white-tailed deer and are beneficial during prolonged and severe winter conditions (Osborn and Jenks 1998). Winters in the Cross Timbers ecoregion are generally mild but can be severe occasionally (Gee et al. 1991). The majority of food plots on the three WMAs are planted in winter wheat (P. Rodamski, B. Burton, R. Umber, pers. comm.). Wheat and rye, when available, rank second in important winter forages for white-tailed deer within the Cross Timbers (Gee et al. 1991). Although Lexington WMA showed a statistical significant difference in fecal $\delta^{13}C$ between years (means: -29.56‰ ± 0.83 and -28.83‰ ± 0.53), it was not considered biologically significant.

The isotopic nitrogen ratio ($\delta^{15}N$) in ungulates, such as white-tailed deer, depends on the amount nitrogen content of their forage (DeNiro and Epstein 1981). Plants acquire nitrogen through bacteria, cyanobacteria, and nodules; however, plants that acquire nitrogen by fixing atmospheric nitrogen ($\delta^{15}N = 0‰$) tend to be less enriched in $\delta^{15}N$ (Virginia and Delwiche 1982, Ambrose and DeNiro 1986, Hobbie et al. 2000, Walter 2006). Plants high in nitrogen are considered high-quality herbivore forage, important for growth, reproduction, etc. (Mattson 1980). Ambrose (1991) found that $\delta^{15}N$ is enriched in the tissues of herbivorous species that forage in open grasslands than the same species foraging in closed forest.

Yearly differences between isotope ratios within the same area may be related to the effects of annual variation in temperatures and rainfall (i.e., increase $\delta^{15}N$ with decreasing precipitation; Cormie and Schwarz 1996, Feranec 2007). Mean fecal $\delta^{15}N$

values did not vary between years at Lexington WMA and Okmulgee GMA; however, mean fecal $\delta^{15}N$ at Cherokee GMA was highly depleted in 2009. Feces of an herbivore are often enriched in ^{15}N over its diet by 0.5–3.0‰ (Sponheimer et al. 2003b). This suggests that positively enriched fecal $\delta^{15}N$ values of white-tailed deer in late winter may represent forages consumed on food plots (e.g, winter wheat, mean $\delta^{15}N$ = 1.96‰; Walter 2006). The positively enriched fecal $\delta^{15}N$ values in 2010 and the negatively depleted fecal $\delta^{15}N$ values in 2009 may be explained by the fact that Cherokee GMA planted fewer food plots in 2009 because of environmental and mechanical conditions (P. Rodamski, pers. comm.). White-tailed deer will increase use of browse when more nutritional forages are not available (Torgerson and Porath 1984, Soper et al. 1993). Browse is depleted in $\delta^{15}N$ (Handley and Raven 1992, Walter 2006) but high in nitrogen content (Botha and Stock 2005). An increase in browse would explain the negatively depleted fecal $\delta^{15}N$ values and high fecal N values at Cherokee GMA in 2009.

Data collected during my study did not support my hypothesis of negative correlations between $\delta^{13}C$ and $\delta^{15}N$. The lack of correlation between fecal $\delta^{13}C$ and fecal $\delta^{15}N$ may be the result differing forage sources of white-tailed deer during winter, and/or the variability of $\delta^{15}N$ in plants within the same species based on nutrient availability to plants and photosynthetic rates (Virginia and Delwiche 1982, Peterson and Fry 1987, Evans 2001, Walter 2006). Differences in nitrogen isotopic ratios may be influenced by rainfall patterns; terrestrial mammals in xeric habitats may have higher $\delta^{15}N$ than in mesic habitats (Handley and Raven 1992, Kelly 2000). Hobson et al. (2000) hypothesized that a correlation between $\delta^{13}C$ and $\delta^{15}N$ may be stronger in species that

consume primarily meat than in species that consume primarily plants because of positive fractionation of isotopic values from one trophic level to another.

Correlations between dietary nitrogen and fecal N concentrations have been demonstrated (Leslie et al. 2008), and high concentrations of fecal N are associated with high-quality diets (Leslie and Starkey 1985, Osborn and Ginnett 2001). The influence of tannins, which bind to proteins forming complexes that are not digestible at the pH of the rumen, has not been shown to be significant in field experiments (Osborn and Jenks 1998, Osborn and Ginnett 2001). Fecal N from the three WMAs did not vary between years or areas (range: 1.76% ± 0.19 – 2.21% ± 0.26). Fecal N values during late winter in the Cross Timbers were comparable to fecal N levels of white-tailed deer in previous studies at similar times of the year (Soper et al. 1993, Osborn and Jenks 1998). Crude protein requirements for maintenance during winter have been estimated at 4–8% for yearling and adult white-tailed deer (French et al. 1956, Holter et al. 1979, Asleson et al. 1996, Berteaux et al. 1998). Fecal N levels in my study were converted to crude protein (fecal N x 6.25 = crude protein) resulting in crude protein dietary levels of 11–13.8% and suggesting reasonably high-quality late-winter diets of white-taild deer at all 3 WMAs.

Diets of white-tailed deer can be nutritionally enhanced in winter as a result of supplemental feeding, e.g., alfalfa hay, winter wheat, and corn (Osborn and Jenks 1998). White-tailed deer will increase their use of food plots during years of mast shortages (Masters et al. 1993). Supplemental feeding within my study areas consisted of food plots provided by the Oklahoma Department of Wildlife Conservation. Fecal N was highest at Cherokee GMA during both years of the study. This is particularly interesting because of differences in fecal $\delta^{15}N$ in which depletion was observed in 2009 and

enrichment was observed in 2010, suggesting differing annual sources of nitrogen during late winter for white-tailed deer. The higher fecal N levels compared to the other WMAs suggested that white-tailed deer at Cherokee GMA received equally, or better, high-quality diets regardless of sources. Fecal N levels at Okmulgee GMA and Lexington WMA were of high quality and only slightly lower quality than Cherokee GMA during both years. A slight decrease in fecal N was observed for all 3 WMAs between years although it was not significant. Yearly differences between fecal N may be result of annual differences in temperatures, precipitation, available soil N levels, and plant nitrogen concentrations (Handley and Raven 1992).

The relationship between fecal $\delta^{15}N$ and fecal N was not strong. The lack of correlation between fecal $\delta^{15}N$ and fecal N was most likely the result of differing nitrogen fixation of plants (Virginia and Delwiche 1982, Ambrose and DeNiro 1986, Handley and Raven 1992). Browse can be high in nitrogen content and depleted in $\delta^{15}N$ (Botha and Stock 2005, Walter 2006), while winter wheat is high in nitrogen content and enriched in $\delta^{15}N$ (Walter 2006). In instances when mixed browsers can eat browse and herbaceous forage within a season, the enrichment of herbaceous vegetation and the depletion of browse can confound attempts at relational studies (Handley and Raven 1992).

Land-cover percentages in the theoretical home ranges showed differences in available habitat for white-tailed deer between areas. White-tailed deer at Okmulgee GMA had available habitats with more percent forest cover and only minimal percent grassland cover. White-tailed deer at Cherokee GMA and Lexington WMA had available habitats with decreased percent forest cover. Cherokee GMA and Okmulgee GMA were generally considered to have more available forest cover than Lexington

WMA. The percentage of grassland cover in theoretical home ranges at Cherokee GMA increased significantly between 2009 and 2010. A significant increase in fecal $\delta^{15}N$ also was observed between years for Cherokee GMA, which may be explained by white-tailed deer having access to more naturally occurring herbaceous plants and food plots in 2010— areas typically more enriched in $\delta^{15}N$ than closed forests (Ambrose 1991).

Prescribed fire is beneficial to the nutrition of white-tailed deer within 3 years post fire in South Dakota (Zimmerman et al. 2006), and alterations to the nutritional quality of white-tailed deer browse are apparent 5–6 years post fire in Oklahoma Cross Timbers (Soper et al. 1993). Available soil nitrogen (NH_4^+ and NO_3^-), important to post-fire recovery of primary productivity and plant growth, increases following fire and NO_3^- does not return to pre-fire levels for 5 years (Wan et al. 2001). Foliar nitrogen also has been shown to increase following fire for at least 4 years (Landsberg et al. 1984). White-tailed deer in the Cross Timbers will select or avoid habitats during particular seasons in response to prescribed burning (Leslie et al. 1996). Fecal samples were collected in late winter when habitat use was most similar for male and female white-tailed deer (Leslie et al. 1996).

Despite wide variation in management practices regarding prescribed fire at the 3 WMAs, white-tailed deer selected comparable home ranges with lowered mean YSF during late winter, resulting in similarities of nutrition among the 3 WMAs at this particular time of year. The mean YSF for the 3 WMAs did not vary (range: 2.0–3.7 years). No relationship existed between mean YSF and mean fecal $\delta^{15}N$ most likely because of variability in plant $\delta^{15}N$ due to differences in nitrogen uptake and available soil nitrogen (Virginia and Delwiche 1982, Peterson and Fry 1987, Handley and Raven

1992, Evans 2001). Differences in nitrogen content may be emphasized when diets are composed of browse and herbaceous plants. No relationship also existed between mean YSF and mean fecal N. The lack of correlations between mean YSF and mean fecal N may be because of similarities in selected home ranges at the 3 WMAs.

Use of stable carbon and nitrogen isotope ratios in combination with fecal N is beneficial in determining nutritional quality and dietary selection in herbivores. Walter et al. (2010) used microhistological analysis in combination with fecal N, $\delta^{13}C$, and $\delta^{15}N$ to distinguish dietary selection and habitat use in relation to cultivated forage availability and found that fecal indices could identify subpopulation structuring of elk. Fecal indices are a convenient non-intrusive method for indexing herbivore nutrition (e.g., Codron et al. 2005, Leslie et al. 2008). Stable isotope ratios of carbon ($\delta^{13}C$) in feces were successful in determining the dominance of C_3 plants in the late-winter diets of white-tailed deer in the Cross Timbers. This was expected because forages common in white-tailed deer diets in the Cross Timbers during late winter and early spring are typically C_3 plants (e.g., browse, winter wheat, and forbs; Gee et al. 1991). Fecal N levels at the 3 WMAs showed that white-tailed deer in the Cross Timbers consumed relatively high-quality diets in late winter. Use of stable nitrogen isotope ratios ($\delta^{15}N$) in feces was useful in showing differing sources of nitrogen in late winter because of depletion and enrichment values in common winter forages of white-tailed deer. Fecal collections in future research should be conducted during multiple times of the year to encompass a variety of forage conditions and determine if relationships exist during other seasons. Because fecal samples were collected opportunistically in my study and no variability in

years since fire was observed, future research should systematically collect feces to elucidate differences caused by fire return intervals.

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Table 1. Means (±SE) for isotopic ratios and nitrogen concentrations of feces collected from white-tailed deer at 3 Wildlife Management Areas in Cross Timbers ecoregion of Oklahoma. Fecal samples were collected opportunistically during February–March, 2009 and 2010. Ratios and concentrations with different letters within a column differed at $P < 0.05$.

AREA	$\delta^{15}N$		$\delta^{13}C$		FN	
2009						
Lexington WMA (n = 8)	2.22 (0.43)	A	-29.56 (0.10)	A	1.87 (0.09)	A
Okmulgee GMA (n = 7)	1.59 (0.73)	A	-30.3 (0.07)	A	1.97 (0.09)	A
Cherokee GMA (n = 10)	-1.80 (0.20)	B	-29.98 (0.21)	A	2.19 (0.15)	A
2010						
Lexington WMA (n = 20)	1.25 (0.30)	A	-28.83 (0.15)	A	1.76 (0.10)	A
Okmulgee GMA (n = 20)	1.98 (0.27)	A	-28.93 (0.41)	A	1.8 (0.11)	A
Cherokee GMA (n = 12)	1.54 (0.27)	A	-29.83 (0.41)	A	2.11 (0.11)	A

VITA

Glen Matthew Hensley

Candidate for the Degree of

Master of Science

Thesis: FIRE EFFECTS ON HABITAT QUALITY FOR WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) WITHIN THE CROSS TIMBERS ECOREGION

Major Field: Natural Resource Ecology and Management, Wildlife Ecology

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Name: Glen Matthew Hensley Date of Degree: December, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: FIRE EFFECTS ON HABITAT QUALITY FOR WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) WITHIN THE CROSS TIMBERS ECOREGION

Pages in Study: 74 Candidate for the Degree of Master of Science

Major Field: Natural Resource Ecology and Management, Wildlife Ecology

Scope and Method of Study: Stable isotope ratios of carbon ($^{13}C/^{12}C$; expressed $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; expressed $\delta^{15}N$) have been used to infer dietary composition of C_3 and C_4 forages and nutritional condition of herbivores. Indices such as fecal nitrogen also can be used to assess dietary quality of large herbivores. The objectives of this study were to assess the effects of varying fire prescriptions on white-tailed deer nutrition and condition in the Cross Timbers ecoregion of Oklahoma. Tissue samples (i.e., bone collagen, incisor enamel, muscle, liver, and hoof) were collected from 257 white-tailed deer during hunts in 2008 and 2009 at 3 Wildlife Management Areas (WMA), 1 State Park, and 4 State-operated check stations (representing private lands). Fecal samples were collected from 77 white-tailed deer in February–March, 2009 and 2010 at the 3 WMAs.

Findings and Conclusions: Mean isotopic carbon ($\delta^{13}C$) values of seasonal representative tissues, i.e., fecal (late-winter), liver (early-winter), muscle (autumn), hoof (summer) and bone collagen and incisor enamel (life-span tissues), suggested that diets of white-tailed deer in the Cross Timbers ecoregion of Oklahoma consisted mostly of C_3 forages. Mean muscle $\delta^{15}N$ increased at the 3 WMAs in the year following the application of fire. Mean hoof $\delta^{15}N$ did not differ between years. Significant increases in mean bone $\delta^{15}N$ were observed at the 3 WMAs as mean years since fire (YSF) decreased in the units in which white-tailed deer were harvested. Mean incisor $\delta^{15}N$ did not differ between years at each WMA, but differences among WMAs did exist. Mean liver $\delta^{15}N$ was significantly lower at Cherokee WMA in both years of this study than Okmulgee GMA and Lexington WMA, and a significant increase was observed at Okmulgee GMA following application of fire. Despite variability in the application of prescribed fire at the 3 WMAs, low values of YSF within theoretical home ranges in combination with levels of fecal $\delta^{13}C$, fecal $\delta^{15}N$, and fecal nitrogen suggested that white-tailed deer at the 3 WMAs selected home ranges with comparable nutrition in late-winter.

ADVISER'S APPROVAL: Dr. David M. Leslie, Jr.